i) using the primers to determine the genomic location of the amplified DNA fragments used. --

REMARKS

Corrections to improve readability were made in the specification on pages 14, 15, 23 and 50. These corrections include the fixing of typographical errors, the moving of a sentence from one location to another, and the completion of a sentence that was inadvertently left incomplete.

Corrections were made on page 25 of the specification to correct citations that inadvertently refer to incorrect points of the patent.

Claims 11 - 83 have been added to fully claim subject matter that was disclosed in the specification.

Claims 1 - 10 have been canceled to avoid duplication.

MARKED SHEET OF THE SPECIFICATION

Page 14, paragraph beginning on line 2:

The protein phytoene desaturase (PDS, encoded by the gene *pds*) is the target of a number of commercially [valuable] available bleaching herbicides. The simple cyanobacterial genetic system, *Synechocystis*, was used to generate and select mutant forms of *pds* resistant to bleaching herbicide 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide. (BASF (Previously American Cyanamid Company), Princeton, New Jersey.)

Page 14, the paragraph beginning on line 17:

The present invention provides a method to isolate and select mutants resistant to 4'-fluoro-6-[(alpha, alpha, alpha,-trifluoro-m-tolyl)oxy]-picolinamide. Two types of mutants may be isolated[,]: spontaneously produced mutants or chemically induced mutants. [Chemically induced mutations may be induced with, for example, ethyl methanesulfonate (EMS).]

Page 15, Paragraph beginning on line 3.

For isolating [EMS] chemically induced mutants, ethyl methanesulfone (EMS) may be used. *Synechocystis* cell cultures were treated with EMS at a concentration which gives a 99% killing rate, followed by growth on selection plates. 100 ~200 ml samples of logarithmic liquid culture were harvested and treated with EMS. The reaction was stopped by addition of sodium thiosulfate, to a final concentration of 5%, to quench excessive EMS. Cells were then collected and washed twice with BG-11. After an overnight recovery in fresh BG-11 medium, cells were plated on solid BG-11 medium containing 1 μM 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide.

Page 23, paragraph beginning on line 8:

Synechocystis [DAN] DNA was prepared using the Qiagen Dneasy Plate Mini Kit (Qiagen, Valencia, California) following NaI pretreatment and digestion with lysozyme as describes in Williams (1988). For manipulation of DNA in *E. Coli*, standard recombination procedures were followed.

Page 16, paragraph beginning on line 3:

In this particular experiment [and for the wild type *Synechocystis* cells], zones of inhibition for the wild type *Synechocystis* cells were observed at the two higher 4'fluoro-6-[(alpha, alpha, alpha,-trifluoro-m-tolyl)oxy]-picolinamide application rates (5×10^{-10} mol and 5×10^{-9} mol) with a diameter of 20 and 38 mm, respectively. However, zones of inhibition were only observed with 4 of the 6 mutants at the highest rate of

4'fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide, results with degree of resistance in the following order: 7-3/11F(0) = 7-4/12F(0) > 5-1/12E(8) > 7-3/12F(12) > 5-1/12F(18) > WT(38) (size of zone in mm in parentheses).

Page 25, Paragraph beginning on line 21:

(1) **Lead compounds identification**: This can be done in a reasonably high through put manner using either the paper disc assay on solid BG-11 agar plate or 96-well microtiter plate as described in Section A and [Examples 3 & Example 4] Example 1.

(2) **Generation and isolation of resistant mutant(s)**: *Synechocystis* mutant(s) resistant to compound of interest can be generated chemically by treating cultures of *Synechocystis* with chemical mutagens (*e.g.* EMS). Procedures for performing such experiment are provided in Examples [6 & 10] 2 & 3.

(3) **Isolation of genomic DNA from resistant cell lines**: Genomic DNA can be prepared from cultures of *Synechocystis* resistance cell lines using commercial kits (e.g. Qiagen [DNAeasy] Dneasy Plant Kit) as [decribed] described in Section B [and Example 7].

(4) **Primer design and PCR amplification of gene fragments from Synechocystis**: Primer pairs for amplification of overlapping DNA fragments from *Synechocystis* can be designed with the assistance of a commercial software package (*e.g.* Vector NTI from InforMax, North Bethesda, MD). Large-scale synthesis of primers can be done by a commercial vendor (e.g. Sigma-Genosys, The Woodlands, TX) in 96-well format. PCR amplification of ~1800 2-kb fragments (*again, the size of the fragment, thus the total number of primers may be altered for easy PCR amplification and HTP manipulation*) can be performed using genomic DNA prepared from mutant cell cultures as template following standard laboratory procedures, as explained in Section B [(Example 9)].

(5) **High Through Put genetic transformation and target site gene identification**: Procedures for HTP genetic transformation and functional complementation assays have been described in Section B [and Example 8]. Gene(s) conferring herbicide resistance can then be identified based on the ability of its PCR products to confer herbicide resistance to wild type cells upon transformation.

Page 50, paragraph beginning line 21:

A further object of this invention provides for cells, tissue, plants, pollen derived from said transformation of the mutant Synechocystis *pds* gene and the *ahas* genes into untransformed plant cells, using the processes mentioned above. Alternatively, mutant forms of *pds* genes with mutation(s) at position(s) similar to the *Synechocystis* gene can be obtained for any given crop species, and used further for genetic transformation. *Synechocystis* mutant *pds* gene(s) resistant to 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide and the mutant AHAS gene comprising the *ahas* small subunit and the *ahas* large subunit identified in these processes can be, respectively, introduced directly into crops for engineering 4'fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]- picolinamide resistance via chloroplast-mediated

transformation and imidazolinone resistance. The genes can also be used for generating resistance to other pds or AHAS inhibiting herbicides.

MARKED SHEET OF CLAIMS

11. An isolated and purified polynucleotide consisting of a mutant *pds* gene from a cyanobacterium, wherein said mutant *pds* gene encodes resistance to 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide.

12. An isolated and purified polynucleotide according to claim 11, wherein said cyanobacterium is selected from the group consisting of *Synechocystis* PCC 6803 and *Anabaena* PCC 7120.

13. An isolate and purified polynucleotide according to claim 11, wherein said mutant *pds* gene has a sequence comprising SEQUENCE ID NO. 3.

14. An isolated and purified polynucleotide according to claim 11, wherein said mutant pds gene encodes cross-resistance to a group consisting of (2E)-2-[amino(benzylsulfanyl)methylene]-1-(2,4-dichlorophenyl)-1,3-butanedione, pyridine, 2-[(3,3-dichloro-2-propenyl)oxy]-4-methyl-6-[[2-(trifluoromethyl)-4-pyrodinyl]oxy] and 1,2,4,5-benzenetetracarboxamide,N,N',N'',N'''-tetrakis[5-(benzoylamino)-9,10-dihydro-9,10-dioxo-1-anthracenyl]. --

15. A replicable expression vector comprising the polynucleotide of Claim 11.

16. A nuclear genome comprising the replicable expression vector of claim 15.

17. A plastome comprising the replicable expression vector of claim 15.

18. A transgenic plant produced from transformation with the replicable expression vector according to Claim 15.

19. A transgenic plant according to claim 18, wherein said transgenic plant exhibits resistance to herbicides selected from the group consisting of 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide, (2E)-2-[amino(benzylsulfanyl)methylene]-1-(2,4-dichlorophenyl)-1,3-butanedione, pyridine, 2-[(3,3-dichloro-2-propenyl)oxy]-4-methyl-6-[[2-(trifluoromethyl)-4-pyrodinyl]oxy] and 1,2,4,5-benzenetetracarboxamide,N,N',N'',N'''-tetrakis[5-(benzoylamino)-9,10-dihydro-9,10-dioxo-1-anthracenyl.

20. Progeny derived from the transgenic plant according to claim 18.

21. A selectable marker for transformation comprising a mutant cyanobacterial *pds* gene containing the polynucleotide of Claim 11.

22. A process for selection for new traits such as herbicide resistance comprising the use of a mutant cyanobacterial pds gene of Claim 11, coupled with the selection on PDS inhibitors.

23. A process for selection for new traits according to claim 22, wherein said PDS inhibitor is 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide.

24. An isolated and purified polynucleotide consisting of a mutant *pds* gene, wherein said mutant *pds* gene has a base pair mutation change of guanine to adenine at position 642 of said mutant *pds* gene.

25. An isolated and purified polynucleotide according to claim 24, wherein said cyanobacterium is selected from the group consisting of *Synechocystis* PCC 6803 and *Anabaena* PCC 7120.

26. An isolate and purified polynucleotide according to claim 24, wherein said mutant *pds* gene has a sequence comprising SEQUENCE ID NO. 3.

27. An isolated and purified polynucleotide according to claim 24, wherein said mutant pds gene encodes cross-resistance to a group consisting of (2E)-2-[amino(benzylsulfanyl)methylene]-1-(2,4-dichlorophenyl)-1,3-butanedione, pyridine, 2-[(3,3-dichloro-2-propenyl)oxy]-4-methyl-6-[[2-(trifluoromethyl)-4-pyrodinyl]oxy] and 1,2,4,5-benzenetetracarboxamide,N,N',N'',N'''-tetrakis[5-(benzoylamino)-9,10-dihydro-9,10-dioxo-1-anthracenyl].

28. A replicable expression vector comprising the polynucleotide sequence of Claim 24.

29. A nuclear genome comprising the replicable expression vector of claim 28.

30. A plastome comprising the replicable expression vector of claim 28.

31. A transgenic plant produced from transformation with the replicable expression vector according to Claim 28.

32. A transgenic plant according to claim 31, wherein said transgenic plant exhibits resistance to herbicides selected from the group consisting of 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide, (2E)-2-[amino(benzylsulfanyl)methylene]-1-(2,4-dichlorophenyl)-1,3-butanedione, pyridine, 2-[(3,3-dichloro-2-propenyl)oxy]-4-methyl-6-[[2-(trifluoromethyl)-4-pyrodinyl]oxy] and 1,2,4,5-benzenetetracarboxamide,N,N',N'',N'''-tetrakis[5-(benzoylamino)-9,10-dihydro-9,10-dioxo-1-anthracenyl.

33. Progeny derived from the transgenic plant according to claim 31.

34. A selectable marker for transformation comprising a mutant cyanobacterial *pds* gene containing the polynucleotide of Claim 24.

35. A process for selection for new traits such as herbicide resistance comprising the use of a mutant cyanobacterial pds gene of Claim 24, coupled with the selection on PDS inhibitors.

36. A process for selection for new traits according to claim 35, wherein said PDS inhibitor is 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide.

37. A selectable marker for transformation comprising a polynucleotide that confers resistance to 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide.

38. An isolated and purified polynucleotide, encoding an AHAS large subunit gene from a cyanobacterium.

39. An isolated and purified polynucleotide according to claim 38, wherein the cyanobacterium is extracted from *Synechocystis* PCC 6803.

40. An isolated and purified polynucleotide according to claim 38, wherein said AHAS large subunit gene confers resistance to an herbicide selected from the group consisting of imidazolinones, sulfonylureas and sulfanylcarboxamides.

41. An isolated and purified polynucleotide according to claim 38, wherein said polynucleotide consists of a sequence comprising SEQUENCE ID NO. 6.

42. A replicable expression vector comprising the polynucleotide of claim 38.

43. A nuclear genome comprising the replicable expression vector of claim 42.

44. A plastome comprising the replicable expression vector of claim 42.

45. A transgenic plant produced from transformation with the replicable expression vector according to Claim 42.

46. Progeny derived from the transgenic plant according to claim 45.

47. A transgenic plant according to claim 45, wherein said transgenic plant exhibits resistance to herbicides selected from the group consisting of imidazolinones, sulfonylureas and sulfanylcarboxamides.

48. A nuclear genome according to claim 43, wherein said replicable expression vector is a construct for nuclear genome transformation comprising an *Arabidopsis* AHAS large subunit promoter and transit sequence, the *Synechocystis* AHAS large subunit coding region, and an *Arabidopsis* AHAS large subunit termination sequence.

49. A selectable marker for transformation comprising a cyanobacterial AHAS subunit containing the polynucleotide of Claim 38.

50. A process for selection for new traits such as herbicide resistance comprising the use of a cyanobacterial AHAS subunit of Claim 38, coupled with the selection on an imidazolinone.

51. A process for selection according to claim 50, wherein said imidazolinone is imazethapyr.

52. An isolated and purified polynucleotide encoding an AHAS small subunit gene from a cyanobacterium.

53. An isolated and purified polynucleotide according to claim 52, wherein the cyanobacterium *Synechocystis* PCC 6803.

54. An isolated and purified polynucleotide according to claim 52, wherein said AHAS small subunit gene confers resistance to an herbicide selected from the group consisting of imidazolinones, sulfonylureas and sulfanylcarboxamides.

55. An isolated and purified polynucleotide according to claim 52, wherein said polynucleotide consists of a sequence comprising SEQUENCE ID NO. 17.

56. A replicable expression vector comprising the polynucleotide of claim 52.

57. A nuclear genome comprising the replicable expression vector of claim 56.

58. A plastome comprising the replicable expression vector of claim 56.

59. A transgenic plant produced from transformation with the replicable expression vector according to Claim 56.

60. Progeny derived from the transgenic plant according to claim 59.

61. A transgenic plant according to claim 59, wherein said transgenic plant exhibits resistance to herbicides selected from the group consisting of imidazolinones, sulfonylureas and sulfanylcarboxamides.

62. A nuclear genome according to claim 57, wherein said replicable expression vector is a construct for nuclear genome transformation comprising an *Arabidopsis* AHAS large subunit promoter and transit sequence, the *Synechocystis* AHAS large subunit coding region, and an *Arabidopsis* AHAS large subunit termination sequence.

63 . A selectable marker for transformation, comprising a cyanobacterial AHAS subunit containing the polynucleotide of Claim 52.

64. A process for selection for new traits such as herbicide resistance comprising the use of a cyanobacterial AHAS subunit of Claim 52, coupled with the selection on an imidazolinone.

65. A process for selection according to claim 64, wherein said imidazolinone is imazethapyr.

66. A rapid plate assay screening method designed to identify inhibitors of specific metabolic pathways, common to photoautotrophic cyanobacteria and higher plants, comprising the steps of:

inoculating cyanobacteria into a simple growth medium;

adding test compounds to the growth medium; and

noting which test compounds inhibit the growth of the cyanobacterium within one to three days.

67. The rapid plate assay screening method according to claim 66, wherein the cyanobacteria are selected from the group consisting of *Synechocystis* PCC 6803, *Anabaena* PCC 7120, and a mixture of *Synechocystis* PCC 6803 and *Anabaena* PCC 7120.

68. A rapid plate assay screening method according to claim 66, wherein the growth medium is 2x BG-11.

69. The rapid plate assay screening method according to claim 66, wherein at least one of the test compounds is 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide.

70. A method to isolate and select mutants resistant to herbicides comprising:

treating algae cell cultures with a chemical that kills the algae cell cultures at a high killing rate;

quenching the chemical reaction with the addition of a second chemical;

plating the surviving algae cell cultures on a solid

medium containing a concentration of said herbicide; and
collecting surviving algae cell cultures.

71. The method according to claim 70, wherein the chemical for creating a chemical reaction is ethyl methanesulfoneate.

72. The method according to claim 71, wherein the second chemical is sodium thiosulfate.

73. The method according to claim 70, wherein the herbicide is 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide.

74. The method according to claim 73, wherein the concentration of 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide is 1uM - 5uM.

75. A method to isolate and select mutants resistant to 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide comprising:
treating algae cell cultures with ethyl methanesulfoneate, which kills the algae cell cultures at a high killing rate;
quenching the chemical reaction with the addition of a sodium thiosulfate;
plating the surviving algae cell cultures on a solid medium containing 1uM - 5uM of 4'-fluoro-6-[(alpha,alpha,alpha,-trifluoro-m-tolyl)oxy]-picolinamide;
collecting surviving algae cell cultures; and
selecting a fragment from herbicide resistant cell lines by using two primers, cgaattccctggtagcatttaatacaattggc and cgcataagctttgcagatggagacggtttgggc.

76. A method for improved genetic transformation of cyanobacteria comprising the steps of:
a) placing competent cyanobacteria into transforming medium in well plates;
b) adding a transforming nucleotide species to the transforming medium;
c) replica plating the cyanobacterium, at least two different time intervals on selection plates containing at least one selection agent.

77. The method according to claim 76, wherein the cyanobacteria are *Synechocystis*.

78. A method for transforming plastomes with cyanobacterial nucleic acid fragments encoding herbicidal resistance comprising the steps of:
a) isolating a cyanobacterial nucleic acid fragment encoding herbicide resistance;
b) incorporating the nucleic acid fragment of step (a) into an expression vector;
c) incorporating the expression vector of step (b) into a plasmid;
d) cutting leaves from a plant and placing them abaxial side down; and
e) bombarding the leaves with the plasmid of step (c).

79. A method for transforming plastomes according to claim 78, wherein the cyanobacterial nucleic acid fragments are derived from a gene encoding a cyanobacterial enzyme selected from the group consisting of a mutant pds enzyme encoding resistance to 4'-fluoro-6- [(alpha, alpha, alpha, -trifluoro-m-tolyl) oxy] - picolinamide, a large AHAS subunit and a small AHAS subunit.

80. The method for transforming plastomes according to claim 78, wherein the expression vector comprises an *Arabidopsis* AHAS large subunit promoter and transit sequence, a *Synechocystis* AHAS large subunit coding region, and an *Arabidopsis* AHAS large subunit termination sequence.

81. The method for transforming plastomes according to claim 78, wherein the plastomes are chloroplasts.

82. The method for transforming plastomes according to claim 78, wherein the plasmids are selected from the group consisting of p116 I, p116 II, p12delta NI, and p12delta NII.

83. A method for target site gene identification in an organism for which a complete genomic sequence is available comprising the steps of:

a) identifying a lead compound which affects the activity of at least one gene of the organism;

b) generating a cell line from the organism which is resistant to the lead compound of step (a);

c) isolating genomic DNA fragments from the resistant cell line of step (b);

d) preparing primer pairs for PCR amplification comprising overlapping DNA fragments from the entire genomic sequence of the organism;

e) amplifying the DNA fragments from the resistant cell line of step (c) by PCR using the primer of step (d) to form amplified DNA fragments from the resistant cell line;

f) transforming competent cells from the organism with the amplified DNA fragments to obtain transformed cells;

g) screening the transformed cells for resistance to the lead compound to obtain resistant transformed cells; and

h) matching the resistant transformed cells to the primers that amplified the DNA used to transform the resistant transformed cells; and

i) using the primers to determine the genomic location of the amplified DNA fragments used.

Respectfully submitted,

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